ASSOCIATION-FORMATION BETWEEN PHOTIC AND SUBTLE GEOPHYSICAL STIMULUS PATTERNS—A NEW BIOLOGICAL CONCEPT[1]

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Living systems have recently been demonstrated to be able to distinguish among strengths and vector directions of magnetic (Brown, 1962a; Palmer, 1963; Picton, 1966), electrostatic (Brown, 1962b), and gamma radiation fields (Brown, 1963; Brown and Park, 1964) of the order of magnitude of those of their natural terrestrial environment. Such responsiveness and a number of its properties have been assayed by quantitative studies of orientational tendencies of organisms as diverse as *Paramecium, Volvox,* planarians, snails, and fruit flies. Demonstrations of such properties as (a) that the maximum capacity of mud-snails to resolve direction of a horizontal magnetic vector occurs at the strength of the local natural one (Brown, Barnwell and Webb, 1964), (b) that effects of brief exposures to magnetic fields deviating slightly from the earth's may persist for many minutes following restoration of the natural field (Brown, Barnwell and Webb, 1964; Brown and Park, 1965a), (c) that a sense of geographic direction in the absence of all obvious environmental cues can be duplicated by a response to experimental horizontal magnetic vectors (Brown, 1962a; Brown, Webb and Barnwell, 1964), and (d) that monthly (lunar day) periodisms in behavior can be abruptly phase-shifted by altering direction of the horizontal magnetic vector (Brown and Park, 1965b), suggested that response to geomagnetism might play some normal role in the lives of the organisms.

In addition, numerous unpublished observations during extensive studies with planarians and mud-snails collectively suggested that organisms possessed some kind of "memory" for geographic directions which did not depend upon any obvious cues. The hypothesis was suggested that the living systems might form associations between their ambient fields of obvious factors and the concurrent pervasive three-dimensional complex of electromagnetic forces of their environment. To test this hypothesis the following series of simple experiments were designed and conducted.

Materials and Methods

About 20,000 *Dugesia dorotocephala* were collected in the field on one day in September, 1965, and were maintained in the laboratory for the duration of the study. They were kept in enameled steel containers with aluminum covers that excluded nearly completely the laboratory illumination. The containers were set in running tap water whose temperature ranged systematically through the year from about 19° C. in July to about 5° C. in February. The worms were exposed to the diffuse laboratory illumination for about 2 hours twice a week while they were fed beef liver.

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Employing apparatus and methods that have been described in some detail in earlier reports (Brown, 1962a, 1963) on planarian orientational tendencies, samples of the worms taken from the stock supply were assayed usually between 9 and 11 AM in each of four types of experimental series. Unvarying were the worms' field of illumination and all other obvious orienting influences in the worms' environment.

The fixed light field for the worms consisted of (1) a point-source directly above the origin of the one-inch test course and hence non-orienting, (2) a horizontal point-source directly behind the initially directed worm, and (3) a horizontal point-source at right-angles to the right of the initial path direction. In this 3-light field the mean path of the test worms always veered to the left, reflecting their negative phototaxis. The degree of the turning was quantified as the angular deviation from straight forward (0°) of the worms' path at the end of a 1-inch free run. The variables were geographic orientation of the whole apparatus and hence initial orientation of the worms, and time. In each of the four types of experimental series, five or six worms were placed in the apparatus at the beginning of the series and were test-run repeatedly until the the series was finished. Then these worms were permanently discarded.

Series IA comprised determining in immediate succession the mean of each of three 15-path samples, requiring about 5 minutes for each sample, for initially North-directed flatworms and then rotating the apparatus with its contained worms *clockwise* by 180° to South and assaying again the mean paths for three immediately following 15-path samples. Series IB involved the same procedure and sequence except that the apparatus was rotated 180° *counterclockwise*. Series IA and IB were carried out first with equal frequency, and fresh worms were always used for the second.

Series IIA involved a fully parallel procedure to IA, except that the worms were assayed first while South-directed followed by 180° clockwise rotation of the apparatus to North and reassay of the worms. Series IIB was like IIA except that the direction of apparatus rotation was counterclockwise.

All experimenters worked with equal frequency with both Series I and Series II. Two different, identically constructed, orientation apparatuses were employed and usually on any given day both a Series I and a Series II were being conducted concurrently. The study was extended rather uniformly over a period of 10 synodic months—from October 8, 1965, through July 29, 1966. By this means all uncontrolled geophysical variables were essentially randomized. The number of series observed for each calendar month was as follows:

		Series I N → S	Series II S → N
1965	1 October (8)	13	11
	2 November	19	16
	3 December	20	20
1966	4 January	26	24
	5 February	28	28
	6 March	23	24
	7 April	21	20
	8 May	20	21
	9 June	16	17
	10 July (29)	21	19
		207	200

The mean turning response was calculated for the three 15-path samples for the initial direction, North or South. Then the mean turning was determined for the first, second and third 15-path samples immediately following the 180° directional change. The values following clockwise rotations were computed separately from those following the counterclockwise ones.

The data for each of the four series for the 10 months were next reduced to mean turning for each day of the synodic month from full moon minus 15 days to full moon plus 15 days, and three-day moving means for these monthly variations were calculated. Such a moving mean was employed to provide a more dependable indication of any systematic variation related to moon phase since each value could be the mean of a sample of 18 to 21 days of data instead of only 5 to 8, and at the same time, appropriately less emphasis would be accorded single monthly-day means which by chance had been based upon a smaller number of days.

Results

In Figure 1 are depicted the mean monthly variations obtained for the worms *initially directed Northward* for the two independent series conducted consecutively on a given day, together with the mean monthly variations of the same two

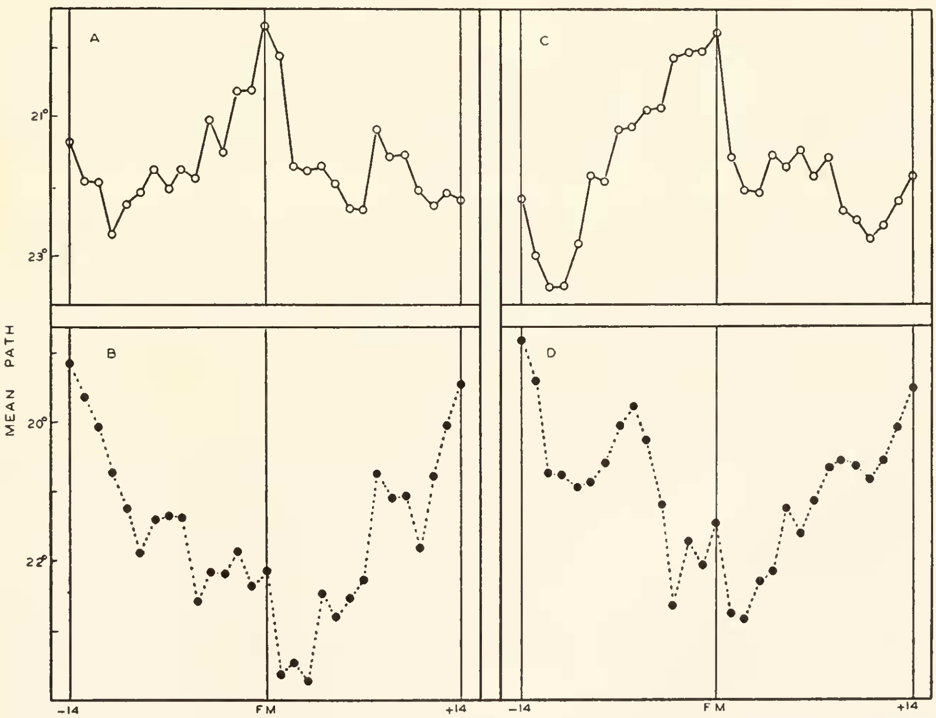

FIGURE 1. A. Mean monthly variation in path of *Dugesia* initially directed *North* with orienting light sources behind and to the right of the worms during a 15-minute assay period. B. Same, immediately after 180° clockwise rotation of the whole apparatus to South. C and D. Repeat of same except with counterclockwise rotation. Ordinate: Degrees of turn to left. Abscissa: Days relative to full moon.

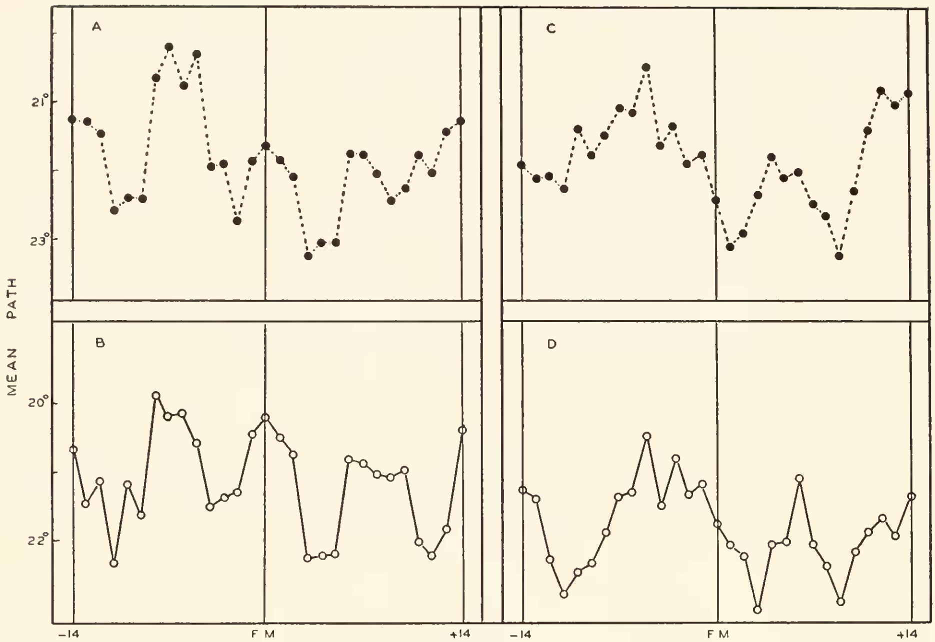

FIGURE 2. A. Mean monthly variation in path of *Dugesia* initially directed *South* with orienting light sources behind and to the right of the worms during a 15-minute assay period. B. Same, immediately after 180° clockwise rotation of the whole apparatus to North. C and D. Repeat of same, except with counterclockwise rotation.

worm-samples after rotation of the apparatus to South, clockwise and counterclockwise. Generally similar to one another are the two independently obtained monthly patterns of variation of the worms while Northbound (Fig. 1, A and C). Quite different, however, are the monthly patterns of variation following the 180° rotation. The two independently derived patterns for these South-directed worms (Fig. 1, B and D) resemble one another rather well despite the opposite directions of the preceding rotation from North to South.

In Figure 2 are shown the comparable monthly patterns for the worms *initially directed Southward* and thereafter rotated to North. Notable in this figure are several things. First, the two independently determined monthly patterns for the South-directed worms (Fig. 2, A and C) are both quite different from the patterns for the South-directed worms immediately following rotation from North (Fig. 1, B and D). They also show some striking differences between them, though for both the maximum for right turning occurs 5 to 7 days *before* full moon and the minimum occurs *after* full moon. But equally evident is an apparent strong tendency for the monthly pattern of variation of the worms after rotation to North to repeat, in general, the same monthly pattern shown by them when previously South-directed (Fig. 2, B and D). Again, as in Series IA and IB, the character of the pattern after rotation appeared independent of the direction of the rotation.

In Figure 3 are plotted the mean monthly patterns, with about a quarter of a cycle repeated, and now centered on new moon. The data for the two series initially North-directed have been averaged together, neglecting direction of rotation, as have also the two initially South-directed ones. In Figure 3A the monthly variation of the South-directed worms following initial North-direction has been temporally displaced by 180°. Evident by inspection is the fact that the worms rotated to South, after North, have a monthly variation of closely the same form

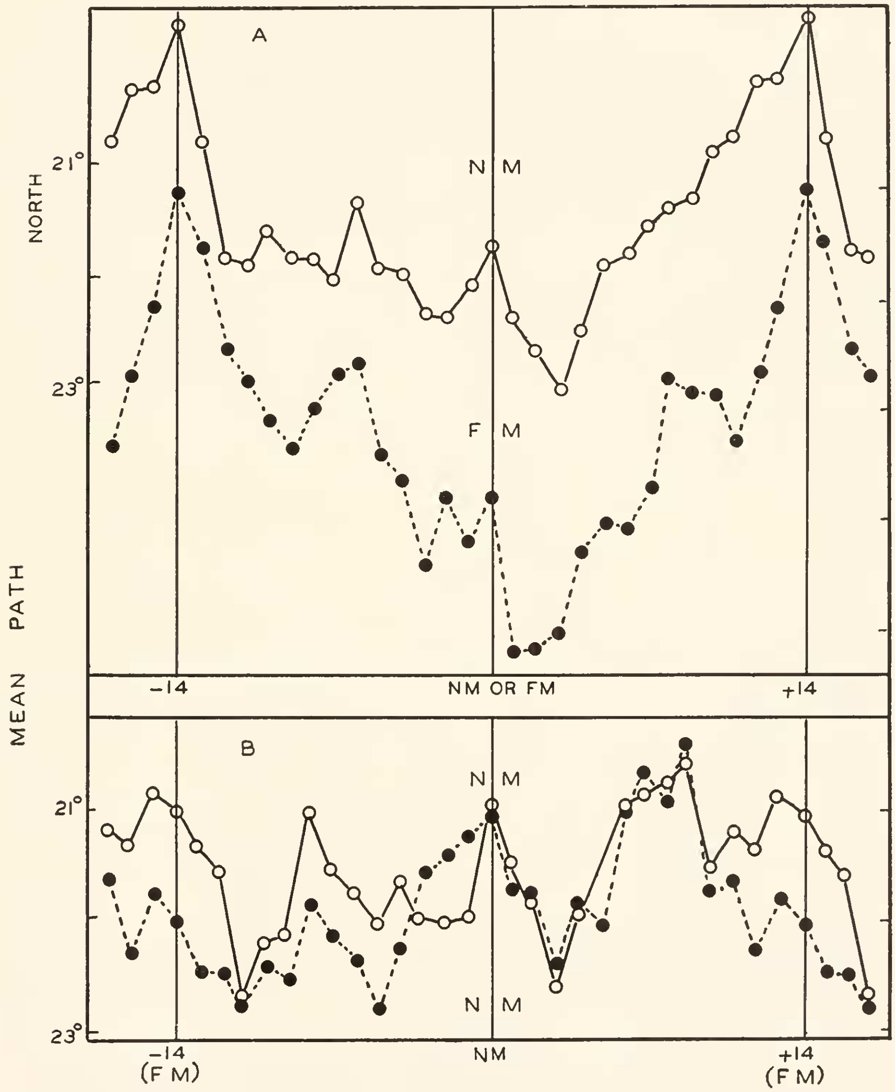

FIGURE 3. A. Combined values for clockwise and counterclockwise rotated worms first North-directed (open circles) and secondly South-directed (dots). The South-directed lunar monthly relationship has been 180° phase-shifted relative to the North-directed. B. The comparable combined data for the worms first South-directed (dots) and then North-directed (open circles).

as that which was present when initially North-directed but has become temporally displaced by 180° and has a slightly greater amplitude. Again is evident the quite different situation for the initially South-directed worms where the pattern shows a strong tendency to repeat the same form and lunar phase relationships after the 180° rotation to North and even to have essentially the same mean amplitude of variation (Fig. 3B).

Discussion

The form and the phase relations of the monthly variation in turning of the initially North-directed worms resemble closely the pattern that has existed steadily over a continuous five-year investigational period (Brown, 1962a, 1963; Brown and Park, unpublished observations). The variation is overt during Autumn and Winter but in Spring and Summer becomes somewhat obscured by greater variance of the samples. It is, however, readily evident during the latter two seasons as mean monthly cycles of the same gross form and phase relations but of significantly decreased amplitude.

The striking phase-shift of the monthly pattern observed after the initially North-directed worms were rotated to South resembles a comparably altered monthly-pattern phase-shift previously reported to follow an abrupt experimental

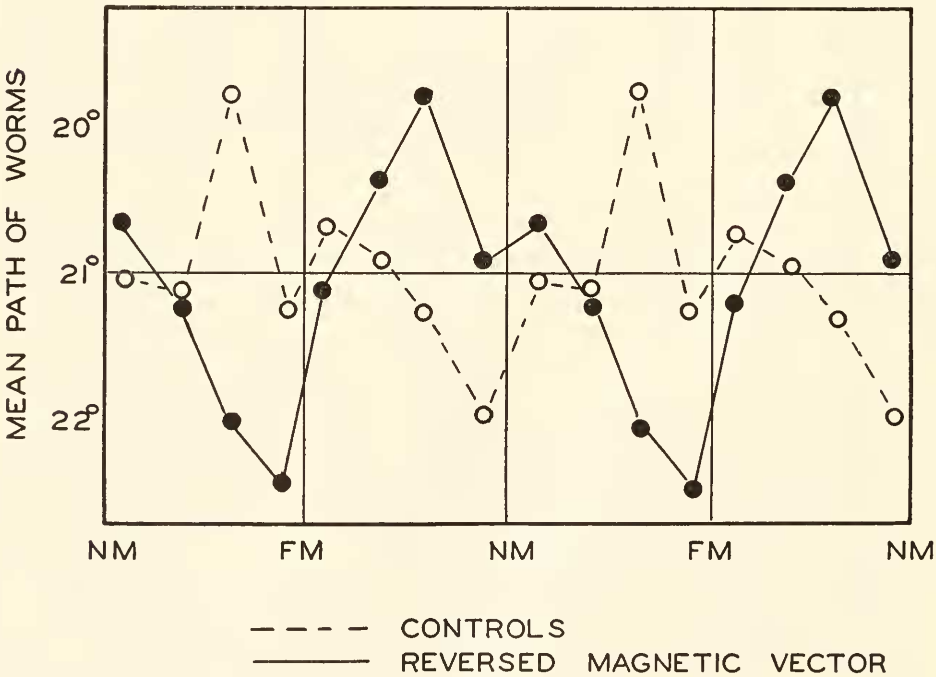

FIGURE 4. The mean monthly variations of North-directed worms during mornings and afternoons in the earth's natural field (open circles) and 30 to 40 minutes after experimental reversal of the horizontal magnetic vector at 0.05 gauss, and, indeed, 15 to 25 minutes after removal of the reversed experimental fields (dots). (Redrawn from Brown and Park, 1965b, to illustrate the 180° temporal phase-shift.)

reversal of the ambient horizontal magnetic vector. When the reversed vector was only about 25% of the strength of the natural geophysical one, the alteration was essentially immediate, but when it was about 25 times the natural field, the alteration was completed only after about 40 minutes of transient states (Brown and Park, 1965b). The latter study had been conducted both mornings and afternoons and the mean monthly cycle-form differed slightly from that obtained for mornings only during the same period. But notable in the results, replotted for comparative purposes in Figure 4, was a very similar 180° temporal shift of the monthly pattern of variation. In neither the present study involving rotation of the apparatus nor the earlier one with reversal of the horizontal vector of magnetism could the results be explained as a simple cycle inversion. In both, the worms appeared to exchange their turning relations with respect to full moon and new moon but at the same time to retain their characteristic asymmetrical cycle-form through what amounted essentially to a 180° cycle-displacement along the temporal axis. In other words, the total monthly pattern was included in the full moon–new moon "exchange." The similarity of the results from these two kinds of experiments, apparatus rotation and magnetic-field rotation, suggests strongly that the chief factor in effecting the results following the 180° rotation of the worms and apparatus in this experiment from North to South is the direction of the ambient horizontal magnetic vector.

The worms are negatively phototactic. While after the rotation of the apparatus from North to South the worms appeared to vary in the strength of their negative phototaxis in a nearly opposite manner, the total explanation cannot be a simple sign-change in phototaxis in view of the detailed asymmetrical pattern participating in the observed alteration.

The worms which were rotated to North into an asymmetrical field with light sources to South and East, after a 15-minute period of residence while South-directed in an asymmetrical field with light sources to North and West, clearly did not respond like worms placed freshly from diffuse illumination into the North-directed apparatus. There must have been an influence of the earlier specific light-magnetic field relationship still persisting after the worms were rotated. And since, comparably, the monthly pattern for South-directed worms with the North and West light sources, after residence for 15 minutes in the asymmetrically lighted field with sources to South and East while North-directed, differed substantially from that of worms placed *initially* in the South-directed field, there must, again, have been a persisting influence of the earlier specific light-magnetic field vector relationship.

It is not known at this time why there should have been the very conspicuous 180° displacement of the characteristic North-directed monthly pattern after the 180° rotation of the worms to South while, on the contrary, there was an equally conspicuous tendency of the worms rotated 180° from South to North to retain not only essentially the same form but also the same phase relationship of their monthly pattern. Hence, while this study indicates the existence of a capacity of the worms to form associations between vector directional components of such overt environmental factors as light on the one hand and such a subtle pervasive geophysical factor as ambient magnetism on the other, and that these associations may persist for at least many minutes, we are still far from a complete understanding of their nature, properties, and biological significance.

From Figure 3A it is seen that after rotation of the apparatus from North to South there appears superimposed on the general 180°-shifted pattern an apparent tendency toward exaggeration of the left-turning behavior over full moon, especially for 1 to 3 days immediately after full moon. This is the time of month of maximum left-turning in the monthly pattern of the worms which are initially South-directed. Correspondingly, in Figure 3B, after rotation of the apparatus from South to North, the major observed difference in otherwise rather similar patterns is an exaggeration of the amount of right-turning over the period of full moon. The last is the normal behavior of the worms when initially North-directed. In brief, these observations suggest that after apparatus-rotation, the mean response pattern for the subsequent period of approximately 15 minutes contains a mixture of two components: (a) a persisting influence of the preceding light-magnetic field vector relationship to which the worms had been exposed, and (b) a characteristic pattern for the worms for that particular geographic direction, other factors equal. Of great interest will be the results of experiments directed toward determining the rate of acquisition of these light-subtle field associations and duration of their persistence.

This study suggests strongly that a living system is able essentially to code an ambient geographic pattern of illumination on a 360° geographic grid of such a subtle geophysical vector field as that of magnetism, and to retain this spatially coded information for at least many minutes. Such a coded geographic "cycle" may provide concurrent alternative, or mutually-supplementing, directional cues, which serve during homing and navigation. The disclosure of such an organismic capacity to code an illumination pattern upon a subtle geophysical variation related to geographic direction provides a basis for postulating that the living system, for any fixed geographic direction, is able to code *temporally* varying information of such an overt factor as illumination on a comparable 360° temporally varying subtle geophysical grid such as that related to the solar day or lunar day. Also supporting such an hypothesis are the gradually accumulating observations that the mechanisms of organismic orientation in time and space possess a common denominator (Brown, 1965). Such an organismic capacity may well serve as a fundamental basis of the phenomena of biological rhythms and clocks and of the apparent clock-dependent astrotaxes.

The "circa" character (not exactly solar-day or lunar-tidal length) of many observed periodisms of animals and plants in unvarying illumination offers no obstacle to an hypothesis that coded, recycling temporal "tapes" are essential, underlying components of the biological clock system, since these odd periodisms may be accounted for as simply a systematic slippage of the cyclic coded patterns along the "tapes" (autophasing).

Summary

1. Evidence is presented that an organism is able to form associations between concurrent ambient vector patterns of light and such a pervasive ambient environmental component as geomagnetism.

2. These associations appear to persist for at least many minutes.

3. Some of the implications of this newly disclosed, extraordinary biological capacity for the still unresolved mechanisms of biological clocks and compasses are discussed.

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